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EXAMINER	
GUNTER, DAVID R	

ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/920,491	DONG, SHOULIAN	
	Examiner	Art Unit	
	David R. Gunter	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-33 is/are pending in the application.

4a) Of the above claim(s) 34 is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-33 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 6-8, 13
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Restriction Requirement

1. Applicant's election of group I in Paper No. 5, received May 15, 2002 is acknowledged. Because applicant did not distinctly and specifically point out any supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)). Upon further consideration of the method claims of groups I-V, the restriction requirement between groups I-V is withdrawn. The restriction between group VI and groups I-V is still deemed proper and is therefore made final. Groups I-V are rejoined and discussed below.

Specification

2. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date as follows: An application in which the benefits of an earlier application are desired must contain a specific reference to the prior application(s) in the first sentence of the specification or in an application data sheet (37 CFR 1.78(a)(2) and (a)(5)). The first line of the specification claims benefit to U.S. application number 09/428,350. This sentence should be amended to include the current status of the parent application (issued as patent number 6,361,947 on March 26, 2002) and the relationship of the current application to the parent (continuation, division, CIP, etc.). Appropriate correction is required.

Oath/Declaration

3. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02. The oath or declaration is defective because it does not properly claim priority to U.S. application 09/428,350.

Claim Objections

4. Claims 31 and 32 are objected to because of the following informalities: claims 31 and 32 recite dependency on claim 27. This dependency of claim 31 on claim 27 is deemed to be improper because claim 31 repeats the limitation recited in claim 27 that "at least one SNP is associated with a disease." It appears that claims 31 and 32 should depend from claim 29, as does claim 30. For the purpose of examination, it is assumed that claims 31 and 32 depend from claim 29, however appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-33 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 1, 20, 25, 29, and 33 are drawn to various methods that all share the common method steps of fragmenting a nucleic acid sample using two restriction enzymes, ligating adapters to the resulting fragments, and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme. The meaning of the term "selectively amplifying" is not clear. In some instances the specification discloses that the method is completely selective, teaching that "[f]ragments with two identical adaptors are not amplified because primer extension is blocked in both strands" (emphasis added; page 16, line 12). In other instances, the specification discloses that the method is only partially selective and that some fragments that were cut on the both ends by the same restriction enzyme will be amplified (figure 1). As broadly as written, the claims encompass any degree of selectivity. The degree of selectivity may be as low as a nearly immeasurable increase in the rate of amplification of the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme compared to the rate of amplification of the fragments cut on both ends by the same restriction enzyme, or the degree of selectivity may be as high as amplification of only the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme without any amplification of the fragments cut on both ends by the same restriction enzyme.

The specification does not offer any teaching that demonstrates the degree to which the amplification of fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme is selected for over the amplification of fragments cut on both ends by the same restriction enzyme. The specification clearly discloses, for example in figure 1, that the fragment cut on both ends with the restriction enzyme designated "RE1" will

undergo at least linear amplification. It is the examiner's assertion that the fragments cut on both ends with the restriction enzyme designated "RE1" will undergo linear amplification only in the first round of PCR amplification, and will then under exponential amplification for the remaining 34 cycles taught by the specification (page 27, line 31).

For the purpose of explanation, the upper strand of the complex formed by the ligation of adapters onto the fragment cut at both ends by the restriction enzyme designated "RE1" (as illustrated in figure 1) will be referred to as strand B and strand B'. Strand B will designate the right-hand portion of the upper strand, including the nucleic acid fragment and the ligated adapter strand comprising and binding site for primer P1. Strand B' will designate the left-hand portion of the upper strand, including the non-ligated strand of the adapter comprising a gap or other structure that prevents ligation of strand B' to the nucleic acid fragment. Similarly, the lower strand of the complex formed by the ligation of adapters onto the fragment cut at both ends by the restriction enzyme designated "RE1" will be referred to as strand C and strand C'. Strand C will designate the left-hand portion of the lower strand, including the nucleic acid fragment and the ligated adapter strand comprising and binding site for primer P1. Strand C' will designate the left-hand portion of the lower strand, including the non-ligated strand of the adapter comprising a gap or other structure that prevents ligation of strand C' to the nucleic acid fragment.

During the first denaturation step, strand B' will dissociate from strand C, and strand C' will dissociate from strand B. During the first hybridization step, strand B will hybridize to strand C with high affinity due to the large number of perfectly complementary bases. Strand C'

will also hybridize to strand B, and strand B' will hybridize to strand C, although with lower affinity.

During the first round of amplification, two distinct types of amplification will take place. In the first type of amplification, primer P1 will bind to the five prime end of strands B and C and synthesize their complements (designated strands A and D, respectively). Synthesis of these complements will include the adapter ligated to the 5' end of the nucleic acid fragment and the entirety of the nucleic acid fragment, but will terminate when the polymerase reaches the end of the nucleic acid fragment due to the gap or other structure that prevents the ligation of strand B' to strand B, and strand C' to strand C. The second type of amplification will occur with a low frequency in the first amplification step. The second type will only take place when strand B' fails to hybridize to strand C, or when strand C' fails to hybridize to strand B. In an instance in which strand B' failed to hybridize to strand C, strand B will act as a PCR primer, and will be extended by the polymerase so that it will comprise a complement to the adapter ligated to the 5' end of strand C. In an instance in which strand C' failed to hybridize to strand B, strand C will act as a PCR primer, and will be extended by the polymerase so that it will comprise a complement to the adapter ligated to the 5' end of strand B. These extended strands B and C will be referred to as "full length fragments."

During the second denaturation step, all DNA fragments will dissociate from one another. With each repetition of the amplification step, there will be a linear increase in the number of strands A and D. However, those B and C strands which were extended to form full length fragments will undergo exponential amplification.

For the sake of discussion, the upper strand of the complex formed by the ligation of adapters to fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme (as illustrated in figure 1) will be designated strand X, X', and X'' to represent the 5' fragment of the "black" adapter which is not ligated to the nucleic acid fragment, the nucleic acid fragment, and the 3' fragment of the "shaded" adapter which is not ligated to the nucleic acid fragment, respectively. The lower strand of the complex will be designated strand Y.

Like the complex formed by ligation of adapters to a nucleic acid fragment cut at both ends by the restriction enzyme designated "RE1," the complex formed by the ligation of adapters to fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme would also undergo only linear amplification during the first amplification step of PCR. Only the strand Y would be amplified because of the gap present between strand X and strand X'. For the purpose of discussion, the newly synthesized complement to strand Y will be designated strand Z. The linear amplification of strand Y to form strand Z can only occur if strand Y does not hybridize to any of strand X, strand X', or strand X''. For example, hybridization of strand X'' to strand Y would block the hybridization of primer P1 to strand Y, and thereby block amplification. The hybridization of either strand X or X' to strand Y would result in premature termination of the strand Z because ligation of strand X or strand X' to strand Z would be blocked. After the formation of a complete strand Z, strand Z can hybridize to primer P2 to begin exponential amplification. Any hybridization of primer P2 to strand X would result only in the synthesis to a complement of strand X.

Based on the above analysis, it would require undue experimentation by a skilled artisan to determine to what degree, if any, the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme are selectively amplified compared to the fragments cut on both ends by the same restriction enzyme. The relative rates of amplification of the two complexes is dependent on several factors:

(1) the ability of strand B, strand C, full length B, full length C and strand Y to compete for primer P1, the primer necessary for the amplification of all of these strands.

(2) the rate at which primer P2 is consumed by amplification strand X prior to the formation of strand Z.

(3) the ability of strand Z and strand X to compete for primer 2.

(4) the degree to which strand X'' and strand B', which are structurally identical, compete with primer P1 for binding to strand B, strand C, full length B, full length C and strand Y.

(5) the effect of the secondary structure of all recited nucleic acid fragments on their tendency to hybridize or to form hairpins or other secondary structures which prevent hybridization. The tendency to form secondary structure will depend on the sequence of the adapters used, the sequence of the genomic DNA, and the restriction enzymes used to digest the genomic DNA.

(6) the temperature and duration of each step of the PCR cycle.

(7) the number of PCR cycles.

The specification offers no guidance to the skilled artisan which would allow them to design adapters or reaction conditions that would result in selective amplification. The skilled artisan would be forced to perform a large degree of undue experimentation in order to determine the temperature and duration for each PCR step, the number of cycles, the restriction enzymes, and the adapters that would allow selective amplification of a particular nucleic acid sample. Because the specification offers no guidance in the determination of these factors, and because of the inherent unpredictability of molecular biology, the skilled artisan would be forced to determine these factors by undue trial and error

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 1-33 are rejected under 35 U.S.C. 112, second paragraph, as failing to set forth the subject matter which applicant regards as their invention.

a. Claims 1-19 are indefinite due to the recitation in claim 1 that the objective of the method is to reduce the complexity of a nucleic acid sample. The method steps of claim 1 recite fragmentation of a nucleic acid, ligation of adapters to the fragments, and amplification of selected fragments. It is not specifically recited that the combination of these steps accomplishes reduction of the sample complexity. The addition of the recitation "whereby complexity of the nucleic acid sample is reduced," or a similar recitation, to the end of the claim would address this rejection.

b. Claims 1-33 are indefinite due to the recitation in claims 1, 20, 25, 29, and 33 of "selectively amplifying." The meaning of the term "selectively amplifying" is not clear. In some instances the specification discloses that the method is completely selective, teaching that "[f]ragments with two identical adaptors are not amplified because primer extension is blocked in both strands" (emphasis added; page 16, line 12). In other instances, the specification discloses that the method is only partially selective and that some fragments that were cut on the both ends by the same restriction enzyme will be amplified (figure 1). The claims should be amended to clarify the meaning of "selectively amplifying."

c. Claims 2-7 are indefinite because the recitation "wherein the amplified fragments comprise at least [a recited percentage] of the first nucleic acid sample" is unclear. It is not clear how the amount of amplified fragments is to be measured, and therefore is unclear how the percentage of amplified fragments is to be determined. For the purpose of examination, the claims will be interpreted to mean that at least a recited percentage of the sequence of the first nucleic acid sample is represented within the plurality of amplified fragments, but the claim should be amended to clarify the meaning of the claim.

d. Claim 11 is unclear due to the recitation "wherein the nucleic acid sample is ... cDNA derived from RNA, total RNA or mRNA." The method of claim 1 recites that the nucleic acid sample is fragmented using "a first and second restriction enzyme," that adapters are ligated to the resulting fragments, and that selected fragments are amplified. It is not clear how single stranded cDNA, RNA, total RNA, or mRNA would be

appropriate substrates for the recited restriction enzymes or ligase. Because these nucleic acids would not be cleaved by the recited restriction enzymes, it is not clear how the subsequent steps of ligation and amplification would take place or how the recited goal of reducing complexity would be accomplished.

e. Claims 12-18 are each indefinite because of the recitation in claim 12 of "wherein ligation of one strand of each adaptor is blocked" and the recitation in claims 13-18 of "wherein ligation is blocked." It is not clear what the strand of the adaptor is being prevented from ligating to, and therefore it is not clear what, if any, structural limitations are being imposed upon the adapter.

f. Regarding claim 18, the claim is indefinite because the meaning of the phrase "the 5' end of one adapter and the 3' end of the other adapter" is unclear. A double-stranded adapter does not have a definite 5' end or 3' end because each end will comprise the 5' terminus of one of the strands and the 3' terminus of the other strand. For the purpose of examination, the claim will be interpreted to be identical to the recitation of claim 17, that ligation is blocked at the 5' end of one strand of one strand of one adapter and at the 3' end of one strand of the other adapter. The claim should be amended to clarify its meaning.

g. Regarding claim 23, the claim is indefinite for the following reasons:

1) The phrase "is designed to query DNA fragments" is unclear. The phrase "is designed to" does not specifically define the structure or function of the array but recites an intended use for the array.

2) The term "query" is indefinite because the nature of the query is undefined.

3) The phrase "DNA fragments which have been produced by the procedures used to obtain the amplified fragments" is redundant. Any DNA fragment is inherently produced by the procedure that produces it.

The claim should be amended to recite specific structural or functional elements of the array and their use in the method of claim instead of reciting an intended use, to clarify the nature of the "query", and to clarify the language concerning the DNA fragments.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 1-8, 10, and 11 are rejected under 35 U.S.C. 102(b) as being anticipated by Smith, PCR Methods and Applications 2:21-27, 1992 (hereinafter "Smith").

a. Claim 1 of the instant application recites a method of reducing the complexity of a nucleic acid sample comprising: fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and

selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme.

Smith teaches a method comprising the steps of fragmenting a nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme (page 21, right column, second paragraph; also figure 1). The specification defines reducing the complexity of a nucleic acid sample as "amplifying a representative subset of the sample" (page 14, lines 1-3). Smith teaches that his method "was originally conceived as a way to generate representative sequencing templates from very large molecules" and that the method can "generate large numbers of unique amplified fragments" (page 25, right column, second paragraph). This use for the method taught by Smith satisfies the applicant's definition of reducing the complexity of a nucleic acid sample.

b. Regarding claims 2-7, these claims recite the additional limitation to claim 1 that the amplified fragments comprise at least a specified percentage of the first nucleic acid sample. The specified percentage ranges from 0.01% (claim 2) to 50% (claim 7). As noted above in item "c" of the section entitled "Claim Rejections - 35 USC § 112," the recitation "wherein the amplified fragments comprise at least [a recited percentage] of the first nucleic acid sample" is unclear. It is not clear how the amount of amplified fragments is to be measured, and therefore is unclear how the percentage of amplified fragments is to be determined. For the purpose of examination, the claims will be

interpreted to mean that at least a recited percentage of the sequence of the first nucleic acid sample is represented within the plurality of amplified fragments.

Smith teaches that use of his method on a 73 kb template results in a plurality of fragments such that "[m]ost parts of the sequence were covered in both directions; 80 regions were covered in only one direction, and 20 gaps ... (accounting for 6% of the sequence) were evident" (page 25, right column, last paragraph through page 26 left column, first paragraph). The teaching that most (i.e. over 50%) of the sequence was covered in both directions, and that gaps between amplified fragments accounts for only 6% of the sequence satisfies the recitation that the amplified fragments comprise at least 50% of the first nucleic acid sample.

c. Regarding claim 8, Smith teaches the embodiment in which one restriction enzyme has a six base pair recognition sequence (Bsp M1, page 22, right column, second paragraph) and the other has a four base pair recognition sequence (Rsa I, page 22, middle column, second paragraph).

d. Regarding claim 10, Smith teaches the embodiment in which the fragments are amplified by PCR (page 23, left column second paragraph).

e. Regarding claim 11, Smith teaches the embodiment in which the nucleic acid sample is genomic DNA (page 22, center column, second paragraph).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over Smith in view of the New England Biolabs, Inc., online catalog (www.neb.com). Claim 1 of the instant application recites a method of reducing the complexity of a nucleic acid sample comprising: fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme. Claim 9 recites the additional limitation to claim 1 that the first restriction enzyme as an eight base pair recognition sequence and the second restriction enzyme has a four base pair recognition sequence.

Smith teaches a method comprising the steps of fragmenting a nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme (page 21, right column, second paragraph; also figure 1). Smith does not specifically teach that the method reduces the complexity of a nucleic acid sample.

The specification defines reducing the complexity of a nucleic acid sample as "amplifying a representative subset of the sample" (page 14, lines 1-3). Smith teaches that his method "was originally conceived as a way to generate representative sequencing templates from very large molecules" and that the method can "generate large numbers of unique amplified

fragments" (page 25, right column, second paragraph). This use for the method taught by Smith satisfies the applicant's definition of reducing the complexity of a nucleic acid sample.

Smith teaches the embodiment in which one restriction enzyme has a six base pair recognition sequence (Bsp M1, page 22, right column, second paragraph) and the other has a four base pair recognition sequence (Rsa I, page 22, middle column, second paragraph), but does not specifically teach the embodiment in which one enzyme has an eight base pair recognition sequence. However, Smith teaches that the size of the fragments generated by the method can be determined by analysis of the nucleic acid template and selection of appropriate enzymes and tags. It was well known to those of ordinary skill in the art at the time the application was filed that digestion of a DNA template with an enzyme having an eight base recognition site will generate a smaller number of fragments than digestion of the same template with an enzyme having a four base recognition sequence (see "Frequencies of Restriction Sites" from the online catalog of New England Biolabs, Inc. (www.neb.com), printed and included with this action). It would have been obvious to one of ordinary skill in the art to modify the method of Smith to incorporate enzymes with an eight base pair recognition sequence in order to produce larger DNA fragments. There are at least two enzymes (Swa I and Pme I; see product data sheets from the online catalog of New England Biolabs, Inc. (www.neb.com), printed and included with this action) that have an eight base recognition sequence and that generate the blunt ends necessary for the method as taught by Smith. It would have been obvious to one of ordinary skill in the art to use either of the enzymes in the method of Smith because of their ready commercial availability, known properties, eight base recognition sequence, and blunt-ended cutting of nucleic acid molecules.

8. Claim 12-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith in view of Makarov and Langmore, USPN 6,197,557, filed September 10, 1998, issued March 6, 2001 (hereinafter "Makarov").

a. Regarding claim 12, Claim 1 of the instant application recites a method of reducing the complexity of a nucleic acid sample comprising: fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme. Claim 12 recites the further limitation to claim 1 that ligation of one strand of each adapter is blocked.

Smith teaches a method comprising the steps of fragmenting a nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme (page 21, right column, second paragraph; also figure 1). The specification defines reducing the complexity of a nucleic acid sample as "amplifying a representative subset of the sample" (page 14, lines 1-3). Smith teaches that his method "was originally conceived as a way to generate representative sequencing templates from very large molecules" and that the method can "generate large numbers of unique amplified fragments" (page 25, right column, second paragraph). This use for the method taught by

Smith satisfies the applicant's definition of reducing the complexity of a nucleic acid sample.

Smith does not specifically teach that ligation of one strand of each adapter is blocked. Makarov, however, teaches fragmentation of nucleic acid samples followed by ligation of adapters in which "[e]ach of these adapters is designed such that the 3' end of the restriction fragment to be sequenced can be covalently joined (ligated) to the adapter, but the 5' end cannot. Thus the 3' end of the adapter remains as a free 3' OH at a 1 nucleotide gap in the DNA, which can serve as an initiation site for the strand-replacement sequencing of the restriction fragment" (column 39, lines 24-31). Makarov teaches that such adapters and subsequent strand-replacement sequencing result "in accurate sequencing reactions ... and thus produce more useful sequence data from large templates" (column 4, lines 30-34). It would have been obvious to one of ordinary skill in the art at the time the application was filed to modify the method of Smith to include the adapters and strand-replacement sequencing as described by Makarov in order to increase the accuracy of the sequencing reactions (Makarov, column 4, lines 30-34).

b. Regarding claim 13, Makarov teaches the embodiment in which ligation is blocked by introducing a gap of at least one nucleotide between one strand of the adapter and one strand of the fragment (column 39, lines 24-31).

c. Regarding claim 14, Makarov teaches the embodiment in which ligation is blocked by the absence of a phosphate at the 5' end of an adapter strand (column 39, lines 43-54).

d. Regarding claim 15, Makarov teaches the embodiment in which ligation is blocked by the presence of a modified nucleotide at the 5' or 3' end of the adapter strand (column 39, lines 43-54).

e. Regarding claim 16, Makarov teaches the embodiment in which ligation is blocked by a terminal modification in one strand of an adaptor (column 39, lines 43-54).

f. Regarding claim 17, Makarov teaches the embodiment in which ligation is blocked at the 5' end of one strand of one adapter and at the 3' end of one strand of the other adapter (column 39, 24-31).

g. Regarding claim 18, as explained above in item "g" under the heading "Claim Rejections - 35 USC § 112," the claim is indefinite because the meaning of the phrase "the 5' end of one adapter and the 3' end of the other adapter" is unclear. A double-stranded adapter does not have a definite 5' end or 3' end because each end will comprise the 5' terminus of one of the strands and the 3' terminus of the other strand. For the purpose of examination, the claim will be interpreted to be identical to the recitation of claim 17, that ligation is blocked at the 5' end of one strand of one strand of one adapter and at the 3' end of one strand of the other adapter

Makarov teaches the embodiment in which ligation is blocked at the 5' end of one strand of one adapter and at the 3' end of one strand of the other adapter (column 39, 24-31).

h. Regarding claim 19, Makarov teaches the embodiment in which adapters produce 5' overhangs and 3' overhangs (column 39, lines 41-53; also table 1 in columns 39-40). These overhangs are taught to be four bases or more in length (column 39, lines 42-43).

As broadly as written, the phrase "overhang comprising a primer binding site" in claim 19 reads on any overhang of sufficient length to bind to any primer at any degree of specificity under any hybridization conditions. Under conditions of sufficiently low temperature and sufficiently high salt concentration, the four base overhangs taught by Makarov will bind to primers, as demonstrated by the ability of the overhangs to hybridize with complementary sequences on the digested DNA template (column 39, lines 41-53).

9. Claim 20-28 and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith in view of Lipshutz, et al., BioTechniques 19(3):442-47, 1995 (hereinafter "Lipshutz").

a. Claim 20 and 25 recite a method of analyzing a nucleic acid sample comprising fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adapters to the fragments; selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme; providing a nucleic acid array; hybridizing the amplified fragments to the array; and analyzing a hybridization pattern resulting from the hybridization.

Smith teaches a method of analyzing a nucleic acid sample comprising the steps of fragmenting a nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme (page 21, right column, second paragraph; also figure 1).

Smith does not specifically teach that the resulting amplified fragments are hybridized to an array.

However, the use of nucleic acid arrays as a means of analyzing nucleic acid fragments was well known to those of ordinary skill in the art at the time the application was filed. Lipshutz, for example, teaches the use of microarrays as a "fast and effective means of accessing ... genetic variation" (page 442, abstract). It would have been obvious to one of ordinary skill in the art at the time the application was filed to modify the method of Smith to include hybridization of the amplified fragments to an array and analysis of the hybridization pattern in order to take advantage of the rapid and effective analysis of the DNA fragments provided by an nucleic acid array.

b. Regarding claims 21, 22, 26, and 33, Lipshutz teaches the embodiment in which the method for analyzing the nucleic acid sample comprises determining whether the nucleic acid sample contains sequence variations (claim 21), and in which the variations are single nucleotide polymorphisms (claim 22, 26, and 33; Lipshutz, page 445, left column)

c. Regarding claim 23, as described above in item "h" in the section titled "Claim Rejections - 35 USC § 112," the claim is indefinite because the phrase "is designed to query DNA fragments" is unclear. The phrase "is designed to" does not specifically define the structure or function of the array but recites an intended use for the array. The term "query" is also indefinite because the nature of the query is undefined. The array described by Lipshutz detects DNA fragments. Therefore, it can be argued that the array

must have been designed to query DNA fragments, and so the limitation of claim 23 is met.

d. Regarding claim 24, Smith teaches the embodiment in which a substantial amount of the sequences predicted to be contained in the amplified fragments are first determined by computer system (Smith, page 25, center column, second paragraph, through right column, third paragraph).

e. Regarding claim 27, Lipshutz teaches the embodiment in which at least one SNP is associated with a disease (page 446, right column, first paragraph).

f. Regarding claim 28, Lipshutz teaches the embodiment in which at least one SNP is associated with the efficacy of a drug (page 446, left column, second paragraph, through center column, second paragraph).

10. Claim 29 is rejected under 35 U.S.C. 103(a) as being unpatentable over Smith in view of Brown and Shalon, USPN 5,807,522, filed June 7, 1995, issued September 15, 1998 (hereinafter "Brown"). Claim 29 recites a method of screening DNA comprising: providing a first nucleic acid sample from each of the individuals; providing a second nucleic acid sample by: fragmenting the nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme; providing a plurality of identical nucleic acid arrays wherein the arrays comprise probes designed to interrogate for DNA sequence variations; hybridizing each of the second nucleic acid samples to one of the plurality of identical arrays; generating a plurality of hybridization patterns

from the hybridizations; and analyzing the hybridization patterns to determine the presence or absence of sequence variation in the population of individuals.

Smith teaches a method comprising the steps of fragmenting a nucleic acid sample using a first and second restriction enzyme to produce fragments; ligating adaptors to the fragments; and selectively amplifying the fragments that were cut on one end by the first restriction enzyme and on the other end by the second restriction enzyme (page 21, right column, second paragraph; also figure 1). Smith does not specifically teach that a plurality of nucleic acid samples are to be provided by a group of individuals, nor does Smith teach hybridizing the amplified fragments to a plurality of arrays.

Brown, however, teaches "a procedure ... to simultaneously screen many patients against all known mutations in a disease gene" using "96 identical ... microarrays" (column 15, lines 19-23). Brown teaches that such an analysis of amplified PCR products (see examples 1 and 2, columns 16-18) allows "significant time and cost savings" compared to "handling, processing and tracking ... 96 separate membranes in 96 separate chambers" (column 15, lines 39-43). It would have been obvious to one of ordinary skill in the art at the time the application was filed to modify the method of Smith to include collection of samples from a plurality of individuals and analysis of the amplified PCR fragments from each individual on an identical microarray in order to screen patients for the presence of a genetic disorder (column 15, lines 19-23) in a cost effective and rapid manner (column 15, lines 39-43).

11. Claims 30-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith in view of Brown in further view of Lipshutz. Claims 30-32 recite the additional limitations to

claim 29 that the sequence variation is at least one SNP (claim 30), that the at least one SNP is associated with disease (claim 31), and that at least one SNP is associated with the efficacy of a drug (claim 32).

Claim 29 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Smith in view of Brown for the reasons cited above in item 11. Neither Smith nor Brown teach the detection of SNPs, or the association of SNPs with disease or drug efficacy. Lipshutz, however, teaches the analysis of nucleic acid fragments which identifies sequence variations including SNPs (Lipshutz, page 445, left column). Lipshutz further teaches that the identified SNPs can be those associated with a disease (HIV infection; page 446, right column, first paragraph) or with the efficacy of a drug (AZT; page 446, left column, second paragraph, through center column, second paragraph).

It would have been obvious to one of ordinary skill in the art at the time the application was filed to further modify the nucleic acid detection method of Smith as modified by Brown in order to detect SNPs known to be associated with HIV infection and drug resistance in individuals infected with HIV. One of ordinary skill in the art would have been modified to use the method of Smith as modified by Brown because of its ability to detect nucleic acid sequences in a cost effective and rapid manner (Brown, column 15, lines 39-43). One of ordinary skill in the art would have been motivated to use this method for the detection of SNPs related to HIV infection and drug resistance in HIV-infected individuals because "there is significant value in being able to rapidly and cost-effectively screen [HIV] genes for mutations" (Lipshutz, page 446, center column, second paragraph).

a. Regarding claim 30, Lipshutz teaches the embodiment in which the sequence variations are single nucleotide polymorphisms (Lipshutz, page 445, left column)

b. Regarding claim 31, Lipshutz teaches the embodiment in which at least one SNP is associated with a disease (page 446, right column, first paragraph).

c. Regarding claim 32, Lipshutz teaches the embodiment in which at least one SNP is associated with the efficacy of a drug (page 446, left column, second paragraph, through center column, second paragraph).

Conclusion

12. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David R. Gunter whose telephone number is (703) 308-1701. The examiner can normally be reached on 9:00 - 5:00 M - F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones can be reached on (703) 308-1152. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 746-9212 for regular communications and (703) 308-8724 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0198.

David R. Gunter, DVM, PhD
January 12, 2003